B015

DIFFERENTIAL MODULATION OF OXIDATIVE AND NITROSATIVE STRESS PATHWAYS BY RED WINE POLYPHENOLS, PROVINOLS™, IN TISSUES FROM ZUCKER FATTY RATS (FA/FA)

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Obesity is associated with numerous complications including significantly increased risks of diabetes and cardiovascular diseases. Epidemiological studies report an inverse association between dietary flavonoid consumption and mortality from cardiovascular diseases. The aim of this work was to study the effects of dietary supplementation of red wine polyphenols extract, Provinols™, on the regulation of both NO and O2- pathways in different tissues in an experimental model of obesity, the Zucker fatty rats (ZF).

Rats received normal diet (n = 6) or supplemented with Provinols™ (20 mg/kg/day, n = 6) for 8 weeks. Then, NO and superoxide anion (O2-) measurement was carried out in heart, lung, and liver by electronic paramagnetic resonance after animals being scarified. Also, tissues were dissected and homogenized for western blot assays protein expression.

	eNOS	p-eNOS S1177	p-eNOS T495	Caveolin-1	g91^{phox}	p67^{phox}	p47^{phox}	Mn SOD	Cu/Zn SOD	EC SOD
Heart	ns	ns	ns	---	ns	---	ns	ns	ns	ns
Lung	+++	ns	ns	ns	ns	+++	ns	ns	ns	---
Liver	+++	ns	ns	ns	+++	ns	ns	ns	ns	ns

Table 1: Western blots analysis of protein expression in tissues from ProvinolsTM-treated rats. Results are expressed as increase (+++) or decrease (---) of protein expression in tissues from ProvinolsTM-treated rats compared to controls. ns, no significant change.

Regarding NO, we found that ProvinolsTM increased its release in both heart and lung, but not in liver. However, ProvinolsTM reduced O2- production in lung and liver without change in heart.

In conclusion, Provinols™ differentially affect the balance between NO and O2-, as well as the associated regulation of protein expression, in tissues from obese rats.

B016

IMPACT OF A 14-NIGHT INTERMITTENT HYPOXIA (IH) EXPOSURE ON METABOLIC AND CARDIOPULMONARY ADAPTATIONS TO EXERCISE IN HEALTHY SUBJECTS

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Introduction — Modifications in exercise tolerance have been reported in obstructive sleep apnea (OSA) patients. Also specific mechanisms have been speculated related to intermittent hypoxia (IH), hypertension, obesity or metabolic disturbance associated to OSA may play a significant role in exercise limitation. In order to eliminate these confounding factors we aimed to evaluate the effects of IH exposure during 14 nights in healthy subjects on exercise capacity, cardio-respiratory response and substrate oxidation during exercise.

Methods — 12 healthy subjects (BMI: 21.8 0.5 kg.m-2) were exposed to repetitive sequences of hypoxia — re-oxygenation during sleep in a hypoxic tent with appropriate cyclic re-oxygenation (rate: 30 desaturations.h-1). Maximal and sub-maximal exercise tests were performed before and after exposure in order to investigate cardio-respiratory variables and substrate oxidation parameters.

Results — IH did not modify maximal exercise parameters (VO2, heart rate, power output) nor ventilatory threshold (VTh). But this was achieved with a significant PETCO2 reduction and a VE/VCO2 increase during both maximal (Pre IH vs Post IH at VTh and Max, $p<0.05$) and sub-maximal (Pre vs Post at 30% and 60% Pmax, $p<0.05$) exercise tests, indicating hyperventilation. At the 1st min recovery after submaximal exercise test, diastolic arterial blood pressure (DBP) was higher after IH exposure (Pre: 60 ± 3 vs Post: 78 ± 2 mmHg) in favour of a delayed DBP recovery following acute exercise. During sub-maximal exercise, subjects reached maximal lipid oxidation at higher power output and presented a decreased blood lactate at the same percentage of relative power after IH exposure.

Conclusion — Exposure to 14 days of nocturnal IH is associated with an increased ventilatory response to subsequent exercise at sea level. Furthermore, delayed DBP recovery after exercise is in favor of early IH-induced cardiovascular modifications. This observation related to muscular exercise adaptations confirms the efficacy of the model in reproducing early cardiovascular alterations occurring in OSAS. Moreover, this model induces metabolic adaptations as soon as 14 nights of exposure.

Jeudi 2 avril 2009, de 10h00 à 11h30

C — STRESS OXYDANT, NO, VIEILLISSEMENT

C001

SENSIBILITÉ DE LA FONCTION ENDOTHÉLIALE AU STRESS OXYDANT CHEZ UN MODÈLE DE RATS EXPOSÉS À UNE POLLUTION DE TYPE CITADINE AU CO

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Objectif — Evaluer les effets d'une pollution de type citadine au monoxyde de carbone (CO) sur la fonction endothéliale, chez une population de rats. Cette évaluation sera réalisée dans des conditions standard, puis consécutivement à un stress oxydant aigu.

Méthodologie — Des rats Wistar (250-280 g) ont été placés dans un environnement simulant une pollution de type urbaine au CO (30 ppm 12 h/jour + 5 pics à 100 ppm) pendant 4 semaines. La fonction vasculaire a été évaluée sur des anneaux d'aorte thoracique isolés et placés dans une cuve à organe. La vasorelaxation endothélium-dépendante a été évaluée sur anneaux pré-contractés par l'ajout de doses croissantes d'acétylcholine en condition standard, puis après un stress oxydant (incubation dans la cuve de peroxyde d'hydrogène, H2O2, 200 µm, 20 mn). Cette incubation a été réalisée en présence ou non d'un inhibiteur spécifique de la NOS2

(SMT 1 µm) ou d'un piégeur du peroxynitrite (Acide urique, 100 µm). La vasorelaxation endothélium indépendante a été évaluée dans l'ensemble des cuves avant et après incubation d'H2O2 par ajout d'une dose de SNP (10-5 m).

Résultats — Dans des conditions standards, 4 semaines d'exposition au CO ne sont pas à l'origine d'une altération de la voie de vasorelaxation NO-cGMP. Il est par contre intéressant de noter, que les rats CO présentent une sensibilité accrue au stress oxydant provoqué par l'incubation d'H2O2 dans les cuves. En effet, les rats CO présentent suite à ce stress oxydant une altération significativement plus marquée de la vasorelaxation endothélium-dépendante (Ctrl rats : 18%; CO rats : 55%) sans modification de la vasorelaxation endothélium indépendante. L'utilisation d'un inhibiteur spécifique de la NOS2 au cours du stress oxydant permet de prévenir les effets aggravants du CO.

Conclusion — Les effets d'une exposition prolongée au CO à des concentrations telles que rencontrées en environnement urbain, sont masqués dans des conditions standards, mais apparaissent majeurs et délétères consécutivement à un stress oxydant aigu au H2O2. La NOS2 semble être impliquée de manière majeure dans les effets délétères du CO.

C002
INCREASE OF AORTIC NITRIC OXIDE PRODUCTION IN METABOLIC SYNDROME

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We demonstrated an increase of nitric oxide (NO) production in aortas of zucker obese fatty rat (ZOF). Metabolic syndrome is associated with an attenuation of acetylcholine (ACh) response. The impairment of ACh induced vasodilation suggests a decrease in nitric oxide (NO) bioavailability. The aim of this work was to assess whether a reduced NO production is involved in the drop of NO bioavailability. Male zucker obese fatty (ZOF, n=8) and lean (n=9) rats, 31 weeks old, were anesthetized and thoracic aortic segments harvested. The NO produced by aortic rings was assayed by EPR spectroscopy using colloid Fe(DETC)2 spin-trapping. The EPR spectra were obtained using a X-Band EPR spectrometer. Basal NO production was significantly (p=0.0001) increase (126%) in ZOF rats (3061±201AU/h/mg) from lean controls (1356±101AU/h/mg). Addition of ACh (3.10-6M) induced an increase in NO levels in aortic rings of both ZOF and lean rats, this relative increase was significantly smaller in ZOF (162±10 vs 270±26%, p=0.002). However the level was higher in ZOF (4905±360 vs 3500±213AU/h/mg, p=0.004). Without endothelium no NO production was detecded in ZOF aortas. A specific inhibitor of neuronal NOS, Nw-Propyl-L-arginine (PLA; 0,1 µm), was unable to decreased both NO productions : the basal NO production and the ACh-stimulated NO production in ZOF aortas. A similar result was obtained with the specific inhibitor of inducible NOS suggesting the only involvement of eNOS in increase of NO production observed in ZOF. We conclude that the vascular dysfunction observed in metabolic syndrome is not due to a decrease in NO production but may be at least in part due to a reduced sensitivity to ACh stimulation.

C003
CYCLOXYGENASE-2 PRESERVES FLOW-MEDIATED REMODELING IN OLD OBESE ZUCKER RATS RESISTANCE ARTERIES

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Resistance arteries have a key role in the control of local blood flow. They are able to remodel in response to chronic increases in flow during growth, exercising or in ischemic diseases. Flow-mediated remodelling is governed by the endothelium. The incidence of metabolic syndrome increases with age and these 2 risk factors reduce endothelium integrity, because of an inflammatory process. We hypothesized that inflammation possibly through the induction of cycloxygenase-2 (COX2), might affect remodeling in old obese rats. In 12-month old obese and lean Zucker rats mesenteric resistance arteries were alternatively ligated in vivo so that one artery was submitted to high flow (HF), compare to normal flow (NF) vessels.

After 21 days, outward hypertrophic remodelling in HF arteries occurred in obese rats (498±20 in HF arteries vs 443±18 µm in NF arteries, P<0.01), not in lean rats (454±17 vs 432±14, NS; n=12 per group). Endothelium-dependent (acetylcholine)-relaxation (AMR) was reduced in obese compare to lean rats. AMR was reduced by NO-synthesis blockade (L-NAME) in all groups and eNOS expression was higher in HF than in NF arteries without difference between lean and obese rats. Indomethacin further reduced AMR in HF from obese rats without significantly affecting arteries from lean animals. As COX2 immunostaining and expression level was evidenced in arteries from obese rats, COX2 inhibition (NS398) was tested on AMR. NS398 significantly reduced AMR in HF arteries in obese rats only. In obese rats chronically treated (3 weeks) with NS398 outward remodelling did not occurred in HF arteries.

Thus, COX2 preserved arterial remodelling in response to a chronic rise in blood flow in old obese rats. This adaptation is in favor of a better tissue perfusion.

C004
CYCLOOXYGENASE-2 INHIBITION RESTORED ENDOTHELIAL-MEDIATED RELAXATION IN OLD OBESE

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End-organs perfusion is altered in metabolic diseases due in part to a decreased endothelium-mediated relaxation in resistance arteries (RA). Inflammatory factors including cyclooxygenase-2 (COX2) derived agents affect the endothelium to different degrees in aging and obesity but the effect of their association on the endothelium is not fully understood. We hypothesized that COX2 derivatives might reduce endothelium-mediated relaxation in aging associated with obesity. RA from 4 and 12 month-old obese Zucker rats RA were isolated to measure acetylcholine (endothelium)-mediated relaxation (AMR), in vitro, using wire-myography.

Impaired with obesity in young rats, AMR was further reduced with aging in old obese rats (89 versus 77% maximal relaxation, young versus old lean rats and 72 versus 51%, old young versus